AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions, and listings, of claims in the application:

1-158. (Cancelled)

159. (Currently amended) A method of detection and/or quantification of a target nucleic acid sequence and/or a nucleic acid amplification reaction using a nucleic acid amplification comprising the step of:

having a Molecular Energy Transfer (MET)/Fluorescent Resonance Energy Transfer (FRET) between a donor MET/FRET moiety and an acceptor MET/FRET moiety separately on at least two separate oligonucleotide[[s]] primers,

wherein the said moieties on two oligonucleotide[[s]] primers are provided in the said oligonucleotide[[s]] primers on a base at least 2 bases away from its 3' end is adapted to inhibit signal from primer dimer, and eliminate non-specific signal generation when the (MET/FRET moiety) labelled oligonucleotide[[s]] primers are subjected to extension by a polymerase in a nucleic acid amplification reaction with the target nucleic acid, and

wherein a MET/FRET signal is generated from donor and acceptor on amplification of said target nucleic acid sequence when the MET/FRET moieties are separated by 4–20 or 25 nucleotides in the amplification product and during amplification.

160. (Currently amended) The method of claim 159, wherein the said primers are 10-40 nucleotides long and adapted for the amplification of a target segment of the size almost close to that of the primer dimer, wherein the length of the primer dimer is close to the length of the forward primer plus the length of the reverse primer plus zero to twenty-five bases

161. (Previously presented) A method of detection of target nucleic acid sequence by nucleic acid amplification reaction as claimed in claim 159, comprising the use of two oligonucleotides as a pair of primers for amplification of said target nucleic acid sequence, with one of them being labeled with a donor/acceptor MET moiety, and a third oligonucleotide labeled with a

complementary acceptor/donor MET moiety of a molecular energy transfer pair, wherein said third oligonucleotide is complementary to the sequence of the labeled primer and is extendable by the polymerase when provided unlinked, and is not extendable when provided linked to the 5' end of the said labelled primer through a non-oligonucleotide organic linker or linker and spacer, wherein both the labeled oligonucleotides are labeled suitably at least two bases away from their 3' ends.

162. (Currently amended) The method of claim 159, wherein a first oligonucleotide primer pair selected to amplify a first segment of the target nucleic acid is used, wherein one of the said oligonucleotide primer pair is provided unlabeled or labeled with a donor or an acceptor MET moiety, a third oligonucleotide primer suitably labeled for MET with an acceptor or donor moiety respectively and designed to amplify a second segment of the first segment being provided such that, the donor moiety is optionally provided in quenched condition with a quencher following conventional methods.

163. (Currently amended) A method for nucleic acid detection or ~~quantitation~~ quantification, wherein an acceptor or donor MET moiety on a labeled oligonucleotide primer is provided in quenched condition with a quencher following conventional methods such that the quencher is capable of absorbing the emission energy of the acceptor or donor and quenching the same, and the donor or acceptor remains quenched only when there is no target amplification and the acceptor or donor labeled oligonucleotide primer~~s are~~ is any one of the labeled primers of claims 159~~-161~~, 160 and 162.

164. (Previously presented) The method of claim 159, wherein both the acceptor and the donor-MET moieties are provided quenched with individual quenchers.

165. (Currently amended) The method of claim 159, wherein a first oligonucleotide primer pair is selected to amplify the target nucleic acid, a second oligonucleotide primer pair is selected to amplify a second segment of the first segment in nested PCR, and said second oligonucleotide

primer pair is ~~any of~~ the two labeled oligonucleotide primers ~~pairs of~~ [[the]] claims 159 and 160, labeled first and third primers of claim 161 and labeled quenched primers of claim 163 and 164.

166. (Currently amended) The method of nucleic acid detection by nucleic acid amplification of claim 159, providing a first oligonucleotide primer labeled with a first MET moiety at least 2 bases away from the 3' end, and a second oligonucleotide primer labeled with a second MET moiety at least 2 bases away from the 3' end, wherein either the first moiety is provided in quenched condition with a third MET moiety following conventional methods of quenching such that the quencher third moiety is capable of absorbing the emission energy of the first moiety, or a third MET moiety is provided that is quenched by said first moiety following conventional methods such that the first moiety is capable of absorbing the emission energy of the third MET moiety only when there is no target amplification where the first and third oligonucleotide are provided unlinked or linked;

wherein on target amplification [[of]], the bases to which the first and the second MET moieties are attached are separated by a distance of 10 base pairs or more in the amplification product, and the same are separated by a distance of 4–20 base pairs resulting in quenching of first or second MET moiety if primer dimer is formed, wherein said first and second ~~label~~ MET moieties are the members of a first MET/FRET pair and said first and the third ~~label~~ MET moieties are the members of a second MET/FRET pair and said first and second MET/FRET pairs are the same or different.

167. (Currently amended) A method of high throughput detection or quantification of target nucleic acid comprising the steps of having a target nucleic acid that carries at it's 3' or 5' end a non-target nucleotide sequence 10–40 nucleotides long, a first amplification primer that is selected from the above non-target sequence, and a second amplification primer that is selected from the target nucleotide sequence, wherein said first and second primers are suitably selected from the labeled primers of claims ~~159-161, 163-164 and 166~~ 160, first and second primer of claim 161, quenched primers of claim 163 and 164 and first and second labeled primers of claim 166.

168. (Currently amended) The method of claim 159, wherein multiple labeled amplification primer pairs are provided for amplification of a multiplicity of target sequences.

169. (Currently amended) The method of claim 159, wherein the first or second oligonucleotide primer is attached or fixed covalently through the 5' end or an internal nucleotide to a solid support through a linker or linker and spacer, and other amplification primers and reagents are provided in an aqueous phase in contact with the said solid phase, the solid support to which the first or second oligonucleotide primer is attached is non-porous and transparent or translucent, glass, ~~glass~~ silicone wafer, plastic or plastic laminated flat surface, tubes or wells of a microtiter plate, or [[a]] silicone wafer, where the plastic selected from the group consisting of polystyrene, polyethylene, silicone and polypropylene.

170. (Currently amended) The method of claim 167, wherein multiples of ~~said~~ second oligonucleotide primer for amplification of multiple target sequences are attached or fixed covalently through the 5' end or an internal nucleotide to a solid support through a linker or linker and spacer, and a common or universal first oligonucleotide primer common for all target sequences and reagents are provided in an aqueous phase in contact with the said solid phase for the detection or ~~quantitation~~ quantification of multiple target sequences in a sample, where the second oligonucleotide primer and the common or universal first oligonucleotide primer are the second and first labeled primers of claims 160, 163 and 166, or the second oligonucleotide primer and the common or universal first oligonucleotide primer are the second and first oligonucleotide primers of claim 161;

wherein said solid support to which the second oligonucleotide primers are attached is non-porous and transparent or translucent, glass, ~~glass~~ silicone wafer, plastic or plastic laminated flat surface, tubes or wells of a ~~microtiter~~ thin walled micro titer plate or [[a]] silicone wafer, where the plastic is selected from the group consisting of: polystyrene, polyethylene, silicone and polypropylene.

171. (Currently amended) The method of claim 159, wherein the label moiety is a MET/FRET moiety, and a MET/FRET moiety is a donor MET/FRET moiety or an acceptor MET/FRET moiety, where the acceptor is selected from radiative fluorophore that gives fluorescence[[;]] and a non-radiative quencher; on illumination with its specific excitation radiation or light the donor moiety emits light or radiation, which is different from the light or radiation of illumination, and the acceptor moiety absorbs the light or radiation or energy emitted by the donor moiety and in turn emits radiation or light which is characteristic of the acceptor moiety and is different from that of the donor moiety as well as the light of illumination;

wherein a non-radiative quencher absorbs the energy or light emitted by the donor but does not emit any light or radiation;

wherein when the two donor and acceptor moieties come within a distance where the acceptor moiety can absorb the energy or emission of the donor moiety there is energy transfer from donor to acceptor, and the acceptor emits energy or light and the donor emission gets quenched, and when the acceptor or quencher is separated from the donor so that there ~~can't~~ cannot be any energy transfer quenching of the donor gets removed and donor is able to emit energy or light;

wherein when more than two FRET moieties are used, different permitted combinations of suitable donor and acceptor moieties are used and a MET/FRET pair is a donor–acceptor pair.

172. (Previously presented) The method of claim 159, wherein the donor and acceptor moieties are selected from any of the known donor/acceptor FRET pairs,

wherein a FRET pair is a combination of a donor and an acceptor moiety such that the absorption spectra of the acceptor FRET moiety overlaps with at least 25% of the emission spectra of the donor FRET moiety, and the donor moiety is selected from the group consisting of fluorescein and fluorescein derivatives, carboxyfluorescein (FAM), coumarin, 5-(2'amino ethyl) amino napthlene – 1 – sulfonic acid (EDANS), rhodamine, anthranilamide, europium and terbium chelate derivatives, a combination of an organic moiety having a large extinction conefficient of absorportion and a fluorophore;

wherein said acceptor moiety is selected from the group consisting of fluorescein, fluorescein derivatives, 2' 7' – dimethoxy 4'5'- dichloro-6-carboxyfluorescein (JOE), ethidium, sulforhodamine 101 (TEXAS RED™), cosin, nitrotyrosine, malachite green, pyrene butyrate, 2-{(E)-3-[1-(5-But-2-ynylcarbamoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihydro-indol-(2E)-ylidene]-propenyl}-1-ethyl-3,3-dimethyl-5-sulfinooxy-3H-indolium (Cy3TM) dyes, 2-{(1E,3E)-5-[1-(5-But-2-ynyl scarbamoylpentyl)-3,3-dimethyl-5-sulfinoxy-1,3-dihydro-indol-(2E)-ylidene]-penta-1,3-dienyl}-1-ethyl-3,3-dimethyl-5-sulfinooxy-3H-indolium (Cy5TM) dyes, 4-(4'-dimethylaminophenylazo)benzoic acid (DABCYL), DABCYL derivatives, rhodamine, rhodamine derivatives, 6-carboxy-X-rhodamine (ROX), N,N,N',N'-tetramethyl-6-carboxyrhodamine (TAMRA), Sulfonyl chloride derivative of sulforhodamine 101 (TEXAS RED™), gold nano particle, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system), and the quencher is selected from the group consisting of DABCYL and its derivatives, rhodamine and its derivatives, gold nano particles, black hole quencher dyes (Azo linked aromatic species with conjugated pi-bonded system).

173. (Previously presented) The method of claim 159, wherein the detection and/or quantitation of amplified target nucleic acid is accomplished by providing double-stranded DNA binding fluorescent dye selected from the group consisting of ethidium bromide, SYBR®Green (2-[2-{(3-Dimethylaminopropyl-propyl)-amino}-1-phenyl-1H-quinolin-(4E)-ylidenemethyl]-3-methyl benzothiazol-3-ium); PICOGREEN® [2-[N-bis-(3-dimethylaminopropyl)-amino]-4-[2,3-dihydro-3-methyl(benzol-2-yl)-methylidene]-1-phenyl quinolinium; ACRIDINE ORANGE (N,N,N',N'-Tetramethyl-acridine- 3,6,-diamine); THIAZOLE ORANGE (1-Methyl-4-[(3-methyl-2(3H)- benzothiazolylidene)-methyl] quinolinium p-tosylate) YO-PRO® 1 (Quinolinium,4-O(((3-methyl-2-(3H)benzoxyazolidene) methyl-1-O3-trimethylaminopropyl)-diiodide) and chromomycin A3 (3B-O-(4-O-acetyl-2,6-dideoxy-3-(C-methyl-alpha-L-arabino-hexopyranosyl)-7-methylolivomycin D) suitable to act as a donor or an acceptor.

174. (Currently amended) The method of claim [[172]] 173, wherein fluoresecin labeled primer and double- stranded DNA binding dye Ethidium bromide are used;

wherein fluorescein acts as a donor and ethidium acts as an acceptor for FRET to take place between the two.

175. (Currently amended) The method ~~for the detection and/or quantification of an amplified target nucleic acid sequence as in~~ of claim 159, wherein one of the primers is labeled with a binding moiety selected from the group consisting of biotin, streptavidin, magnetic particle, microsphere, a hapten, an anchor oligonucleotide linked directly or linked through a linker to the said primer; and

wherein there is provided a capture moiety attached to a well of a microtiter plate or a tube or a glass wafer for capturing the respective binding moiety, wherein the capture moiety is selected from streptavidin, biotin, magnet, anti-hapten antibody, a capture oligonucleotide;

wherein the microsphere is captured by a capturing process like certrifugation;

wherein a suitable fluorescent intercalating dye or a suitable fluorescent dye labelled nucleotide capable of acting as donor or acceptor is provided, or a primer labeled with a binding moiety selected from biotin, streptavidin, hapten, microsphere at least two bases away from 3' end and another primer labeled with fluorescent dye or luminescent rare earth metal chelate, fluorescent or gold nano particle, streptavidin, biotin or hapten and suitable conjugate and substrates are provided.

176. (Currently amended) The method of claim 159, wherein said nucleic acid amplification reaction comprises any known nucleic acid amplification reactions ~~including~~ and polymerase chain reaction in particular comprising the steps of adding a polymerase or polymerases, reaction buffer, deoxynucleoside triphosphates in addition to the effective amounts of amplification primers and other oligonucleotides and reagents to the sample, carrying out an initial denaturation followed by repeated cycles of a denaturation step and a selective annealing step, or repeated cycles of a denaturation step, a selective annealing step and an extension step, and an optional final extension step, exciting the reaction mixture with a donor exciting radiation or light, measuring the emission of an acceptor FRET moiety, or that of the donor.

177. (Previously presented) The method of claim 159, wherein said oligonucleotide primers are linear oligonucleotide or duplex oligonucleotide in which a complementary oligonucleotide is joined to the 5' end of the priming oligonucleotide with a non-oligonucleotide organic linker or a linker and a spacer and are selected from the group comprising DNA, RNA or chimeric mixtures, derivatives or modified versions thereof adapted for hybridizing and priming nucleic acid amplification reaction, and are deoxy oligonucleotides, oligonucleotide or peptide or locked nucleic acid or modified oligonucleotides (contains modified base, sugar or backbone); the target nucleic acid sequence is selected from genomic DNA, mRNA, RNAs, cDNA, amplification product, chemically or biochemically synthesized DNA or RNA.

178. (Currently amended) The method of claim 159, wherein the nucleic acid amplifications are a polymerase chain reaction (PCR), or a reverse transcription PCR (RT-PCR), or an allele specific PCR, or a methylation status PCR, or an in situ PCR, or a Triamplification, or an isothermal amplification reaction, ~~including~~ comprising Nucleic acid sequence based amplification (NASBA), or Strand displacement amplification, or an immuno PCR.

179. (Previously presented) The method of claim 159 wherein the target nucleic acid sequence is one of:

an amplification product or the sequence of an infectious disease agent,

a genomic sequence of a human, animal, plant or any other living organism, a mutation in which is implicated to the presence of a disorder or disease,

a human, animal or plant genomic sequence, the presence or absence of which is implicated to a disorder or disease,

a human, animal or plant genomic sequence, the presence or absence of which is implicated to susceptibility to an infectious agent,

a human, animal, plant or any living organism genomic sequence the presence or absence of which is implicated to a genetic trait or genotyping of human, animal, plant, or the living organism,

a genomic sequence of an infectious agent, the presence or absence of which is implicated to strain typing, or

a sequence of a gene a mutation of which is related to a particular allele of the gene.

180. (Previously presented) The method of claim 159, wherein for the heterozygous mutation detection which comprises two amplification primer oligonucleotides, one labeled with a donor MET moiety near 3' end and the other being labeled with an acceptor MET moiety near 3' end, wherein a target amplification reaction and a thermal denaturation analysis of the amplification product or products thus amplified is carried out and in the same method, the labeled oligonucleotide primers are also being provided in dual labeled quenched primer configuration.

181. (Currently amended) The method of claim 159, wherein the first and second oligonucleotides are selected from the group consisting of:

~~Seq Id 10: 5' GGG GTA CTA CAG CGC CCT GA 3'~~

Seq Id 19: 5'- GGG GTA CTA CAG CGC CCT GA –3'
FAM

~~Seq Id 13: 5' GTC CTG GAA GAT GGC CAT GG 3'~~

Seq Id 20: 5' – GTC CTG GAA GAT GGC CAT GG – 3'
JOE

~~Seq Id 12: 5' ATG GCC ATC GTC CTG GAA GAT GGC CAT GG 3'~~

Seq Id 22: 5' – DABCYL-ATG GCC ATC GTC CTG GAA GAT GGC CAT GG – 3'
JOE

Seq Id 23: 5'– DABCYL-ATG GCC ATC GTC CTG GAA GAT GGC CAT GG –3'
FAM

Seq Id 24: 5' – GCT CAT GGC GCC TGC CTG G – 3'
DABCYL

~~Seq Id 11: 5' ATG GCC ATG TCC TGG AAG ATG GCC ATG G 3'~~

~~Seq Id 21: 5' GGG GTA CTA CAG CGC CCT 3' Seq Id 21~~
~~|~~
~~FAM~~

Seq Id 25: 5' – GTC CTG GAA GAT GGC CAT GG – 3'
|
Rhod

Seq Id 26: 5'- GTC CTG GAA GAT GGC CAT GG – 3'
|
JOE

~~Seq Id 29: 5' GGC AAT GAA AAG CCA CTT CT 3' as a forward primer to amplify a 50 base pair segment (base position 23, 565-23, 614) of E.coli genome; and~~

~~Seq Id 30: 5' TTA ACC GGC GAT TGA GTA CC 3' as a reverse primer to amplify a 50 base pair segment (base position 23, 565-23, 614) of E.coli genome.~~

182. (New) A method of high throughput detection or quantification of target nucleic acid comprising the steps of having a target nucleotide sequence that carries at it's 3' and 5' ends two additional non-target nucleotide sequences 10–40 nucleotides or more long, where the said non-target nucleotide sequence can be same or different,

and a first amplification primer is selected from either of the two additional non-target sequences and a second amplification primer that is selected from the target sequence, wherein said first and second amplification primers are suitably selected from the labeled primers of claims 160, first and second primers of claim 161, quenched primers of claim 163 and 164, and first and second labeled primers of claim 166;

further a first and a second amplification primers are selected from the two non-target sequences where the first and second amplification primers are the labeled first and second primers of claim 166, and in case of analysis of multiple targets one of the non target sequence

is common and the other non-target sequence is different for different targets, further a first and a second amplification primers are selected from the two non-target sequences and a third amplification primer is selected from the said target sequence where the said first, second and third primers are the first, second and third primers of claim 162.

183. (New) A method of claim 161 wherein the second oligonucleotide primer is provided labeled with a donor or an acceptor moiety and one or both of the first and third oligonucleotide primers are labeled with an acceptor or donor moiety respectively where second and third primers hybridize to the same one strand of target and a DNA ligase is also provided, further additionally the first oligonucleotide primer is provided unlabeled and is either complementary or not complementary to the third oligonucleotide primer.

184. (New) A method of claim 159, wherein the polymerase is a DNA polymerase or a Reverse transcriptase or a DNA polymerase with reverse transcriptase activity or a DNA polymerase with or without strand displacement activity or a DNA Polymerase or thermostable polymerase.

185. (New) A method of claim 162, wherein a first oligonucleotide primer of the first oligonucleotide primer pair is provided labeled with a donor or an acceptor moiety and the third oligonucleotide primer is labeled with an acceptor or donor moiety respectively where first and third labeled primers hybridize to one strand of target and a DNA ligase is also provided.